Carbohydrate Polymers 92 (2013) 534–539

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Antimicrobial N-halamine modified chitosan films

Rong Li[a], Pei Hu[a], Xuehong Ren[a,*], S.D. Worley[b], T.S. Huang[c]

[a] Key Laboratory of Eco-textiles of Ministry of Education, College of Textiles and Clothing, Jiangnan University, Wuxi, Jiangsu 214122, China
[b] Department of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849, USA
[c] Department of Poultry Science, Auburn University, Auburn, AL 36849, USA

ARTICLE INFO

*Article history:*
Received 12 May 2012
Received in revised form 30 August 2012
Accepted 31 August 2012
Available online 7 September 2012

*Keywords:*
Chitosan
N-halamine
Antibacterial
Biocidal
Films

ABSTRACT

The inherent antimicrobial properties and biodegradability of chitosan make it an ideal candidate for antimicrobial materials. In this study, N-halamine precursor 3-glycidyl-5,5-dimethylhydantoin (GH) was synthesized and bonded onto chitosan by a ring opening reaction between chitosan and GH. The chitosan film modified with the N-halamine precursor could be rendered biocidal after exposure to a dilute household bleach solution. Syntheses routes, characterization data, and antimicrobial test results are presented. The chlorinated films with $2.60 \times 10^{18}$ atoms/cm$^2$ of active chlorine were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895) and showed good efficacy against these two bacterial species with log reductions of 7.4 and 7.5 within 10 and 5 min of contact time, respectively. These films may serve as potential materials for food packaging and biomedical applications.


## 1. Introduction

Recently, there has been a growing interest in the development of antimicrobial films in various applications including biomedical surface coatings and food packaging. The antimicrobial packaging films have shown great potential to control growth of food borne pathogens, including *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Salmonella typhimurium* (Cagri, Ustunol, & Ryser, 2004). Extensive research work has been performed to develop antimicrobial packaging to inactivate the pathogens and spoilage microorganisms on the surface of food (Moura, Mattoso, & Zucolotto, 2012; Tankhiwale & Bajpai, 2012). One of the simplest and most feasible methods of producing antimicrobial films is to load antimicrobial agents, such as nanoparticles (Au, Ag, Cu, ZnO, $TiO_2$) (Fayaz, Balaji, Girilal, Kalaichelvan, & Venkatesan, 2009; Li, Xing, Jiang, Ding, & Li, 2009; Sikong, Kongreong, Kantachote, & Sutthisripok, 2010), essential oils (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011), bacteria originated antibacterial proteins (bacteriocins) (Seydim & Sarikus, 2006), enzymes (Buonocore et al., 2003), fruit extracts (Du et al., 2008), and chitosan (Aider, 2010; Tripathi, Mehrotra, & Dutta, 2010) onto films. However, the release of these additives from the films, especially water-soluble compounds and metal nanoparticles, may raise issues regarding adverse environmental problems and their safe use in food products. The addition of these compounds might also affect the film properties and food qualities.

Chitosan, the linear and partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan (Muzzarelli et al., 2012), is a biopolymer obtained from chitin. It is the second most abundant polysaccharide in nature after cellulose, and has received great attention because of its non-toxicity, biocompatibility, versatility, biodegradability, and antimicrobial properties (Xu, Xin, Li, Huang, & Zhou, 2010). Because chitosan has intrinsic antimicrobial properties and good film-forming ability, it has been used in antimicrobial films and coatings to inhibit the growth of not only Gram-positive and Gram-negative bacteria, but also yeasts and molds (Chen, Yeh, & Chiang, 1996). However, the antimicrobial activity of chitosan is only moderate which may limit its many applications. In order to improve its antibacterial activity, numerous modifications of chitosan have been reported such as N-carboxybutylation (Muzzarelli et al., 1990; Muzzarelli, Ilari, & Petrarulo, 1994), O-carboxymethylation (Chen & Park, 2003), quaternization (Sajomsang, Ruktanonchai, Gonil, & Warin, 2010), sugar-modification (Sajomsang, Gonil, & Tantayanon, 2009), N-alkylation (Yang, Chou, & Li, 2005), and chitosan/nanoparticle complexes (Li, Deng, Deng, Liu, & Li, 2010).

For over three decades, N-halamine compounds have gained growing attention as antimicrobial agents due to their efficacies against a broad-spectrum of microorganisms, long-term stabilities, non-toxicity to humans, and regenerabilities upon exposure to aqueous free chlorine solutions (Worley et al., 2003; Kocer, Cerkez, Worley, Broughton, & Huang, 2011). N-halamines refer to compounds that contain amine, amide, and imide halamine bonds, which have the capability of rapid and total inactivation of various

* Corresponding author. Tel.: +86 051085912007; fax: +86 051085912009.
*E-mail address:* xhren@jiangnan.edu.cn (X. Ren).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.08.115

microorganisms without causing the microorganisms to develop resistance to them. Furthermore, N-halamine biocides are capable of killing microorganisms directly without the release of free chlorine into the system (Barnes et al., 2006). These N-halamines could be incorporated into or attached onto textiles, medical devices, and other solid surfaces related to health care (Worley, Chen, Wang, & Wu, 2005). The introduction of the N-halamines in textiles by covalent bonding between the precursor moieties and host polymers has been extensively explored in these laboratories and elsewhere over the past decade (Liang et al., 2007; Luo & Sun, 2008). N-halamine moieties have been applied onto cotton by using grafting techniques or other coating methods to produce antimicrobial cellulose (Sun & Sun, 2001), nylon (Lin, Cammarata, & Worley, 2001), and polyester (PET) (Ren et al., 2008).

However, little research has been done on the modifications of chitosan by covalently incorporating N-halamine moieties to produce biocidal chitosan. Recently, Cao and Sun (2008) reported that antimicrobial chitosan was prepared by exposure of chitosan to dilute sodium hypochlorite. The N-halamine chitosan provided total kill of $10^8$–$10^9$ CFU/mL of *E. coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria) in 10 and 60 min, respectively. The physical state of chitosan is a crucial factor affecting antimicrobial activity, and extensive works have been done for chitosan in water solution. Little attention has been paid to the investigation of inactivation of microorganisms by chitosan in the solid state, such as for films and fibers. In this study, an N-halamine precursor 3-glycidyl-5,5-dimethylhydantoin was synthesized and attached to chitosan by a ring-opening reaction. The newly synthesized N-halamine chitosan derivative was characterized by FT-IR, NMR, DTG, and TGA. The chitosan derivative was dissolved in acetic acid solution and coated onto polyester transparency slides and then rendered antimicrobial upon chlorination. The chlorinated films were evaluated for biocidal efficacies against both Gram-negative bacteria *E. coli* O157:H7 and Gram-positive bacteria *S. aureus*.

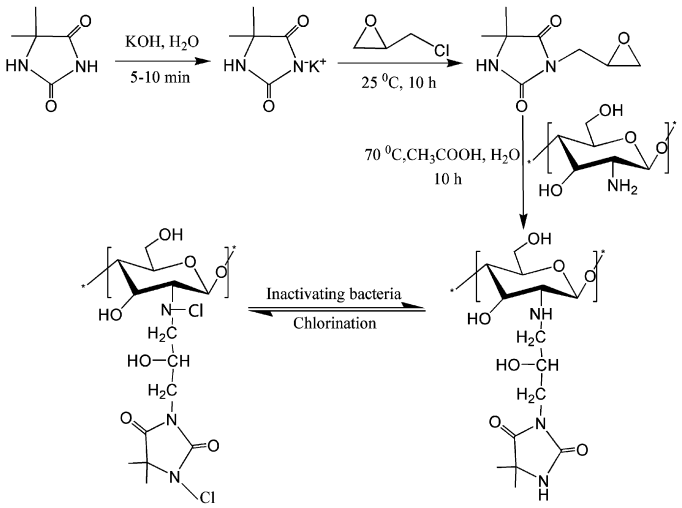
## 2. Experimental

### 2.1. Materials

Chitosan with a molecular weight of 200 kDa and deacetylation degree of 95% was purchased from Zhejiang Aoxing Biochemical Co., Ltd., China. 5,5-Dimethylhydantoin was purchased from Hebei Yaguang Fine Chemical Co., Ltd.; epichlorohydrin, acetone, household bleach (the active chlorine content was 5%), and acetic acid (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai. All reagents were used as received without further purification. The PET film was purchased from Wenzhou Kewen Teaching Equipment Co., Ltd. The bacteria employed were *S. aureus* ATCC 6538 and *E. coli* O157:H7 ATCC 43895 (American Type Culture Collection, Rockville, MD). The Trypticase soy agar used was from Difco Laboratories, Detroit, MI.

### 2.2. Instruments

The FT-IR spectra of chitosan, chitosan–GH and chitosan–GH–Cl were recorded by a Nicolet Nexus 470 spectrometer in the optical range of 400–4000 $cm^{-1}$ by averaging 32 scans at a resolution of 4 $cm^{-1}$. All samples were prepared as potassium bromide pellets. The UV–VIS spectra of chitosan, GH, and chitosan–GH were measured by a UNICO UV-2802S in the range of 190–900 nm. The $^1H$ NMR of GH and $^{13}C$ NMR spectrum of chitosan–GH's was recorded on a Bruker AV-300 spectrometer. Thermogravimetric analysis (TGA) was carried out using a TGA unit (NETZSCH STA 499 F3). About 5 mg of sample was heated from 20 °C to 600 °C at a heating rate of 5 °C $min^{-1}$ under nitrogen atmosphere (flow rate of 40 mL $min^{-1}$).




**Scheme 1.** The schematic description of synthesis of chitosan–GH and chitosan–GH–Cl.

### 2.3. Synthesis of 3-glycidyl-5,5-dimethylhydantoin (GH)

3-Glycidyl-5,5-dimethylhydantoin (GH) was synthesized using the method reported by Liang et al. (2007). In brief, an equimolar mixture of NaOH and 5,5-dimethylhydantoin were added to water and stirred for 5–10 min at ambient temperature. Then an equimolar quantity of epichlorohydrin was added followed by stirring for 10 h at ambient temperature. After the reaction water was removed at reduced pressure, and acetone was added to the flask to dissolve the product. Sodium chloride produced in the reaction was removed by filtration, and the desired product was obtained by the evaporation of the solvent acetone. The yield of the product was 89.23%. $^1H$ NMR (d-acetone): $\delta$ 1.41 (6H), 2.77–2.91 (2H), 3.20 (1H), 3.48–3.65 (2H).

### 2.4. Modification of chitosan with GH

Appropriate amounts of GH and chitosan (the molar ratios of GH to chitosan were 0.8, 1.0, 1.2 and 1.5) were dissolved in 1% (v/v) acetic acid solution. The mixture was stirred at 65 °C for 10 h. Water was removed under vacuum, and excess acetone was added to the flask. The precipitate was filtered and washed with abundant acetone, then dried in a vacuum oven at 60 °C for 48 h. The yields of the products were about 93%. The syntheses of GH and chitosan–GH are shown in Scheme 1.

### 2.5. The degree of substitution of chitosan by GH

The degree of substitution (DS) of chitosan by GH was measured by UV–VIS spectroscopy; the absorbance of different concentrations of GH were employed in the standard curve. DS is defined as the molar ratio of bonded GH of glucosamine calculated from the original mass of chitosan and its degree of deacetylation (DD). The absorbance of chitosan–GH acetic acid solution was measured first, and then the corresponding concentrations were found from the standard curve ($c_2$). The DS were determined by the following equation:

$$DS = \frac{c_2}{(C_1 - c_2 \times M_2/M_1) \times DD} \times 100\% \quad (1)$$

where $c_2$ was the concentration of GH grafted on chitosan, mol/L; $C_1$ was the concentration of chitosan–GH, g/L; $M_2$ was the molecular weight of GH; $M_1$ was the monomer average molecular weight of chitosan; and DD was the degree of deacetylation of chitosan, respectively.

### 2.6. Preparation of chitosan–GH films

The synthesized chitosan–GH was dissolved in 2% acetic acid solution at different concentrations (from 1% to 6%). Then 10 mL of polymer solution were coated on transparency PET slides (9 × 9 cm), followed by evaporation of the solvent in the fume hood overnight. The polymer films were neutralized by immersing in 1 mol/L aqueous NaOH solution at ambient temperature under constant stirring for 1 h, followed by thoroughly washing with deionized water. After air drying, the polymer films were stored in a desiccator for further characterization and testing.

### 2.7. Chlorination procedure

The chitosan, chitosan–GH and chitosan–GH films were soaked in a 5% solution of household bleach (pH adjusted to 7 using 1 N $H_2SO_4$) with stirring at ambient temperature for 60 min. After chlorination, the samples were washed thoroughly with deionized water to remove free chlorine. The chlorinated films were dried at 45 °C for 1 h and stored in a desiccator for further testing.

### 2.8. Analytical titration

The iodometric/thiosulfate titration procedure was employed to determine the oxidative chlorine loading of the modified compounds and the coated films. For example, about 0.05 g of chlorinated sample was suspended in a 50 mL water solution, into which, 0.5 g of KI and 1 mL of 1% of starch water solution were added. The solution was titrated with 0.005 N sodium thiosulfate. The bonded oxidative chlorine on the chitosan–GH compounds was calculated with the equation below:

$$Cl^+\% = \frac{N \times V \times 35.45}{W \times 2} \times 100 \quad (2)$$

where $N$ and $V$ are the normality (equiv./L) and volume (L) of the $Na_2S_2O_3$ consumed in the titration, respectively, while $W$ is the weight of the chitosan and chitosan–GH–Cl samples in grams.

The chlorine loadings of the films (the amount of chlorine on the surfaces) on the slides were calculated with the following equation:

$$Cl^+(\text{atoms/cm}^2) = \frac{N \times V \times 6.02 \times 10^{23}}{2 \times S} \quad (3)$$

where $N$ and $V$ are the normality (equiv./L) and volume (L) of the titrant sodium thiosulfate, respectively, and $S$ is the area of the film in $cm^2$.

### 2.9. Antimicrobial test

Both chlorinated and unchlorinated modified chitosan films were challenged with *S. aureus* (ATCC 6538) and *E. coli* O157:H7 (ATCC 43895) using a modified AATCC Test Method 100-1999. The testing began with the addition of 25 L of the bacterial suspensions buffered at pH 7 to the centers of 6.45 $cm^2$ portions of slides in a sterile Petri dish, and with second identical slides placed upon the first ones held in place by a sterile weight. Different slides were exposed to the bacteria with contact times of 1, 5, 10, and 30 min, respectively; they were placed in tubes containing 5.0 mL of sterile 0.02 N sodium thiosulfate to remove all oxidative chlorine. Series of the quenched solutions were diluted with pH 7, 100 mM phosphate buffer, and plated on Trypticase soy agar. The plates were incubated at 37 °C for 24 h. The bacterial colonies were recorded and enumerated for biocidal efficacy.

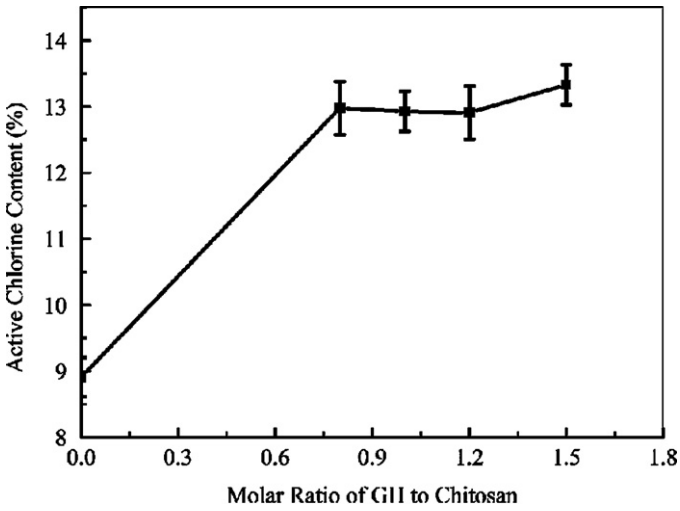



**Fig. 1.** The effect of molar ratio of GH on active chlorine content in chitosan–GH–Cl.

## 3. Results and discussion

### 3.1. The effect of molar ratio of chitosan and GH on the chlorine loadings

The degree of substitution (DS) of chitosan by GH was measured by UV–VIS spectroscopy; the absorbances of different concentration of GH were employed in constructing a standard curve. In this study, four different DS of chitosan–GH were synthesized by changing the molar ratio of GH to chitosan from 0.8 to 1.5. The DS increased from 3.5% to 4.6% by the increasing of the molar ratio.

The effects of molar ratio of GH and chitosan on the active chlorine content of the chitosan–GH samples are shown in Fig. 1. The molar ratio of GH to chitosan ranged from 0.8 to 1.5 (DS was 3.5%, 4.1%, 4.6% and 4.5%, respectively). Fig. 1 shows that the increase of molar ratio within the above range does not significantly affect the oxidative chlorine content. The increase of the molar ratio of GH could not further increase the active chlorine content which confirms that the amine groups in chitosan are favorable sites for the reaction; this was in agreement with that of the DS measurement. A molar ratio of 1:1 was employed in this study. However, the active chlorine loading of chitosan after the introduction of GH was significantly increased from 8.9% of chitosan to 12.9% of chitosan–GH indicating that the amide nitrogen atoms on the hydantoin ring were being chlorinated.

### 3.2. Preparation of chlorinated chitosan–GH films with different concentrations

Different concentrations of the synthesized chitosan–GH, 1%, 2%, 4%, 6%, were dissolved in 2% acetic acid solution. The chitosan–GH solution was coated on PET transparency slides. After neutralization and drying, the films were chlorinated according to the method mentioned in experimental section. The chlorine loadings of chitosan–GH on these transparency slides are shown in Fig. 2. The active chlorine contents increase from $2.15 \times 10^{18}$ atoms/$cm^2$ to $2.60 \times 10^{18}$ atoms/$cm^2$ within the range from 1% to 4%, while further increase of concentration of chitosan–GH (increased to 6%) does not significantly increase the active chlorine contents. Thus, 4% of chitosan–GH solution was used for coating the films in further studies. Films with high chlorine loading of $2.60 \times 10^{18}$ atoms/$cm^2$ would be expected to be quite antibacterial.

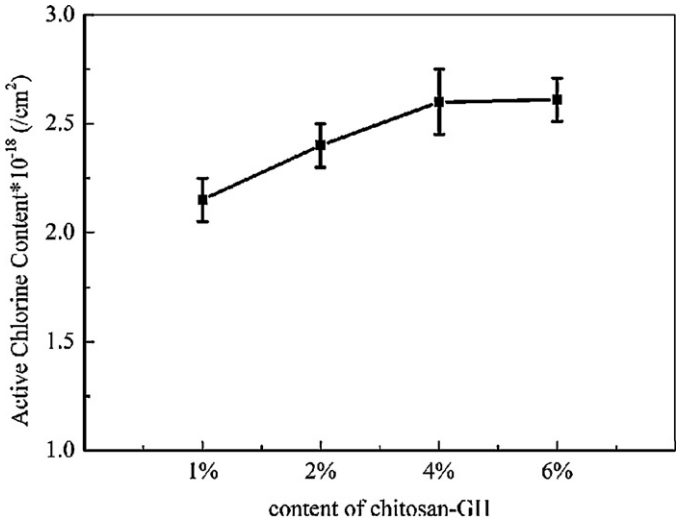



**Fig. 2.** Effect of the concentration of chitosan–GH on active chlorine content.

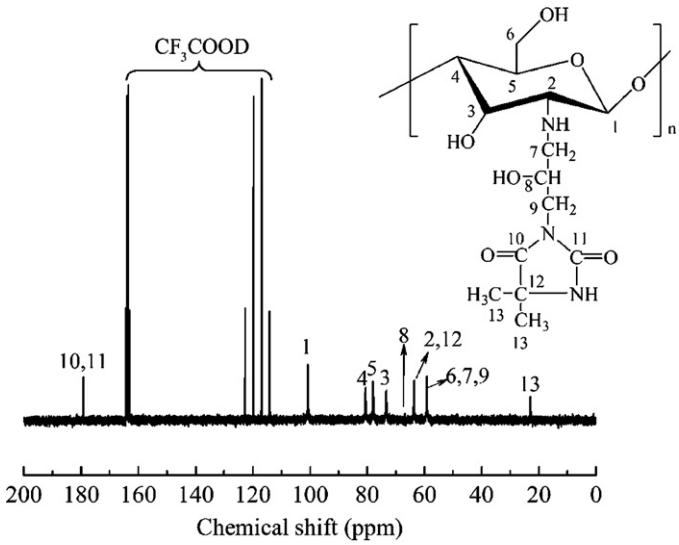



**Fig. 4.** The $^{13}C$ NMR spectra of chitosan–GH using $D_2O$ and $CF_3COOD$ as the solvents.

### 3.3. Characterization

#### 3.3.1. Structural determination by $^{13}C$ NMR, FT-IR and UV–VIS

The FT-IR spectra of chitosan, chitosan–GH and chitosan–GH–Cl are recorded in Fig. 3. The spectrum of chitosan showed the characteristic absorption bands at 1655 (amide I, C=O stretching), 1597 (amide II, $-NH_2$ bending) and 1381 $cm^{-1}$ ($-CH_2$ bending). Other characteristic bands of the polysaccharide appeared at 1152 $cm^{-1}$ (symmetric stretching of the C–O–C) and 1080 $cm^{-1}$ (skeletal vibration of the C–O stretching) (Sajomsang, Gonil, & Tantayanon, 2009). The strong band at around 3400 $cm^{-1}$ was assigned to the stretching vibration for O–H, the extension vibration of N–H, and inter-molecular hydrogen bonds of the polysaccharide moieties. All of the bands mentioned above were common in the spectra of chitosan–GH samples due to the presence of the chitosan backbone. Compared with the FT-IR spectrum of pure chitosan, the spectra of chitosan–GH showed two strong absorption peaks at 1569 $cm^{-1}$ (C=O stretching) (Haro et al., 2005), which indicates that chitosan was successfully modified with GH through covalent bonds. The vibrational band of C=O shifts from 1569 $cm^{-1}$ to 1630 $cm^{-1}$ after chlorination. The shifts to higher wave number of the hydantoin carbonyl bands upon chlorination have been reported previously in the literature (Ren, Kocer, Worley, Broughton, & Huang, 2009).

The $^{13}C$ NMR spectrum of chitosan–GH in $D_2O$ and $CF_3COOD$ solution is presented in Fig. 4. The signals ascribed to the carbons in the chitosan backbone at 100.7 ppm (C-1), 63.6 ppm (C-2), 73.2 ppm (C-3), 80.6 ppm (C-4), 78.0 ppm (C-5), and 59.2 ppm (C-6) are shown in Fig. 4 (Jiang et al., 2011). The signals at 56.9 ppm, 68.1 ppm, 63.6 ppm, 179.2 ppm, and 22.9 ppm may be assigned to the carbons of the $-CH_2$ (C-7, C-9), –CH (C-8), –CH (C-12), –C=O (C-10, C-11), and $-CH_3$ (C-13) (Lu, Wu, & Fu, 2007; Ren et al., 2009) groups, respectively. These signals on the GH units detected above further confirmed that GH was bonded to chitosan successfully.

The UV–VIS spectra of chitosan, GH, and chitosan–GH are shown in Fig. 5. The UV absorption at 240 nm can be attributed to the hydantoin ring structure (Chen & Sun, 2006), which is evident in the spectra of GH and chitosan–GH. Chitosan does not show absorption under UV analysis. The band at 240 nm of chitosan–GH provided further evidence that the hydantoin ring structure was covalently bonded onto chitosan.

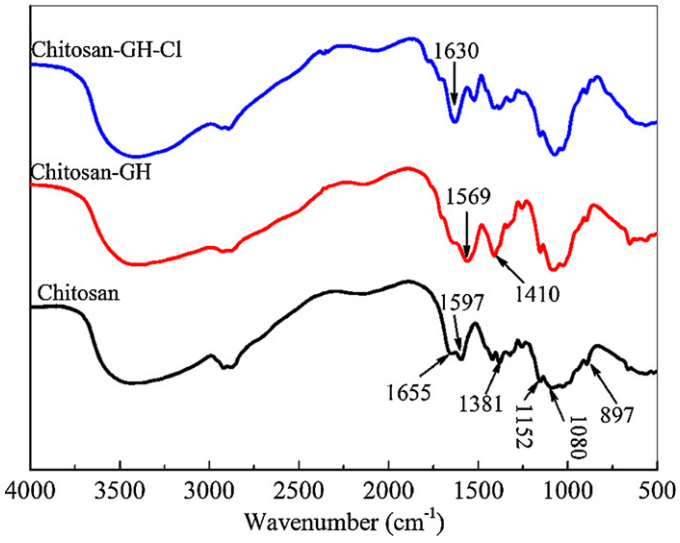



**Fig. 3.** The FT-IR spectra of chitosan, chitosan–GH and chitosan–GH–Cl.

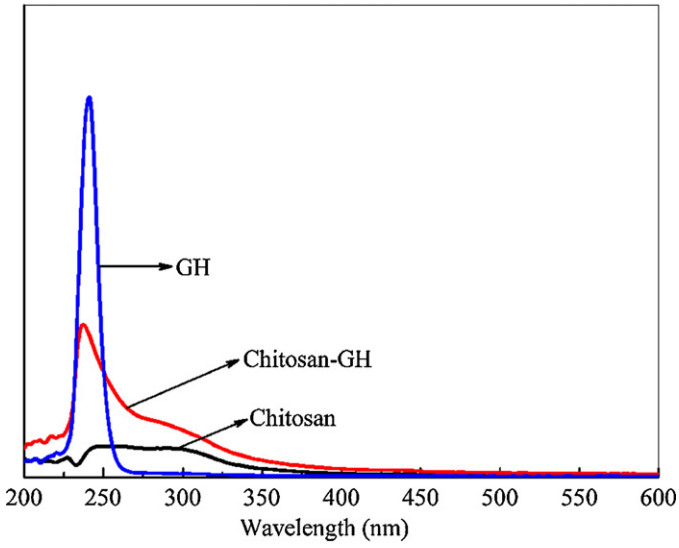



**Fig. 5.** UV spectra of chitosan, GH, and chitosan–GH.

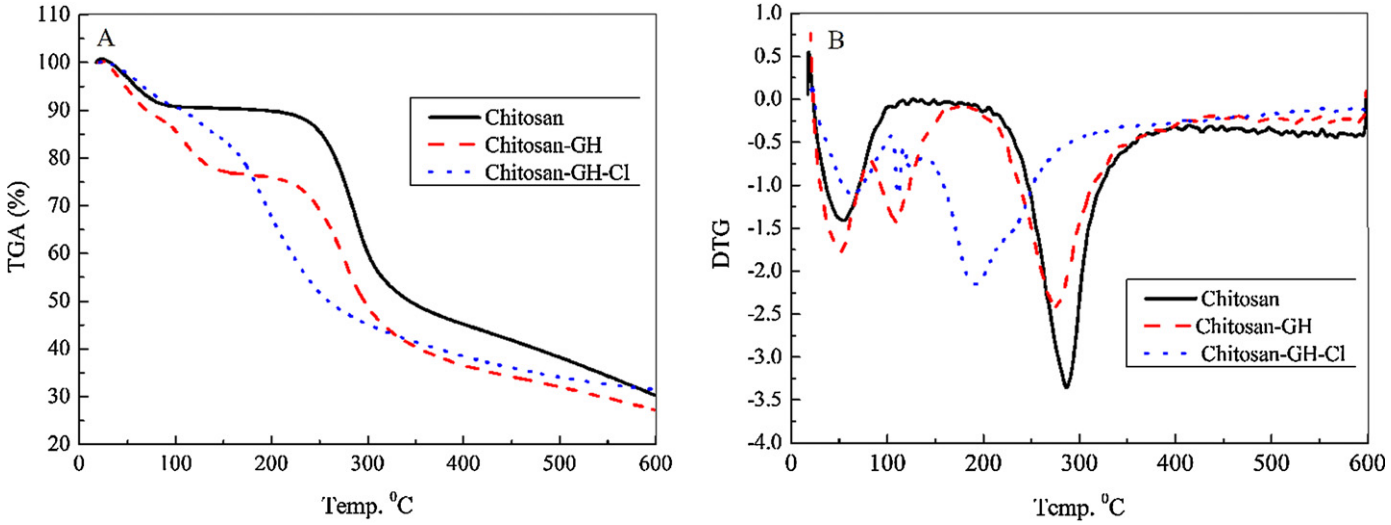



**Fig. 6.** TGA and DTG curves for chitosan, un-chlorinated chitosan–GH and chlorinated chitosan–GH.

**Table 1**
Antibacterial property against *S. aureus*[a] and *E. coli* O157:H7[b].

| Sample | Contact time (min) | Bacterial reduction *S. aureus*[a] | | Bacterial reduction *E. coli* O157:H7[b] | |
|---|---|---|---|---|---|
| | | % | Log | % | Log |
| Un-chlorinated chitosan–GH Film | 30 | 39.34 | 0.22 | 3.97 | 0.02 |
| Chlorinated chitosan–GH Film | 1 | 99.90 | 2.98 | 99.62 | 1.52 |
| | 5 | 99.93 | 3.14 | 100 | 7.48 |
| | 10 | 100 | 7.39 | 100 | 7.48 |
| | 30 | 100 | 7.39 | 100 | 7.48 |

[a] The inoculum population was $2.43 \times 10^7$ cfu/sample.
[b] The inoculum population was $3.73 \times 10^7$ cfu/sample.

### 3.3.2. Thermal analysis

The TGA thermograms of chitosan, chitosan–GH, and chitosan–GH–Cl are shown in Fig. 6. The TGA of chitosan showed two different stages of weight loss. The first stage weight loss extended from 32 to 101 °C, and $T_{max}$ was located at 51 °C. This may correspond to the loss of adsorbed and bound water (Kumar, Koh, Kim, Gupta, & Dutta, 2012). The second stage of weight loss starts at 217 °C and continues up to 350 °C ($T_{max}$ was located at 286 °C) and is due to the degradation of chitosan biopolymer (Kumar et al., 2012). Adelaida Ávila et al. also reported that the weight loss between 40 °C and 200 °C may be attributed to the adsorbed and bound water (Ávila, Bierbrauer, Pucci, López-González, & Strumia, 2012). Therefore, the weight loss of chitosan–GH extending from 28 to 80 °C ($T_{max}$, 51 °C) and from 80 to 156 °C ($T_{max}$ was located at 108 °C), and the weight loss of chitosan–GH–Cl extending from 28 to 101 °C ($T_{max}$ was located at 62 °C) and 101 to 118 °C ($T_{max}$, 108°C) also may be attributed to the adsorbed and bound water. The weight loss of chitosan–GH from decomposition from 200 to 360 °C ($T_{max}$, 275 °C) is very close to that of chitosan, which indicates that incorporation of GH in chitosan does not significantly affect its thermal performance. The chlorinated chitosan–GH–Cl showed that the weight loss temperature from 145 to 280 °C ($T_{max}$, 192 °C) is lower than that of the un-chlorinated chitosan. The decomposition of the N–Cl bonds in the chlorinated chitosan–GH may accelerate the thermal decomposition of chitosan–GH through a free radical process.

### 3.4. Antibacterial efficacy

The biocidal efficacy data for the unchlorinated and chlorinated chitosan–GH films against Gram-positive *S. aureus* and Gram-negative *E. coli* O157:H7 are presented in Table 1. The films were challenged with *S. aureus* and *E. coli* O157:H7 bacteria at $2.43 \times 10^7$ cfu/sample and $3.73 \times 10^7$ cfu/sample, respectively. The unchlorinated chitosan–GH films produced only a small log reduction for both bacteria, 0.218 log reduction of *S. aureus* and 0.017 log reduction of *E. coli* after 30 min of contact due to the adhesion of the bacteria to the films and perhaps a small degree inactivation of the bacteria. The chlorinated chitosan–GH films containing $2.60 \times 10^{18}$ atoms/cm$^2$ of active chlorine completely inactivated both *S. aureus* and *E. coli* O157:H7 with log reductions of 7.39 and 7.48 within 10 min and 5 min of contact, respectively. About 99.9% of both *S. aureus* and *E. coli* O157:H7 could be killed by the chlorinated films within 1 min of contact.

## 4. Conclusions

N-halamine chitosan (chitosan–GH) was prepared by a ring-opening reaction between chitosan and GH. The incorporation of GH into chitosan was confirmed by FT-IR, $^{13}$C NMR, UV, and TGA analyses. The active chlorine content of chitosan–GH–Cl and chitosan–GH–Cl film could reach 12.9% and $2.60 \times 10^{18}$ atoms/cm$^2$, respectively, upon exposure to dilute household bleach. The N-halamine chitosan provided excellent antimicrobial efficacies against both Gram-negative and Gram-positive bacteria by inactivating 7 logs of *S. aureus* and *E. coli* O157:H7 within 5–10 min. The addition of stable cyclic N-halamine with a hydantoin ring into chitosan significantly increases the active chlorine loadings on the films which enhances the antimicrobial activity and durability. These encouraging results show that N-halamine-based chitosan has potential for a wide range of biomedical applications including

wound dressings, coatings for medical devices, and food packaging.

## References


Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry: Review. *Lwt-Food Science and Technology*, *43*, 837–842.

Ávila, A., Bierbrauer, K., Pucci, G., López-González, M., & Strumia, M. (2012). Study of optimization of the synthesis and properties of biocomposite films based on grafted chitosan. *Journal of Food Engineering*, *109*, 752–761.

Barnes, K., Liang, J., Wu, R., Worley, S. D., Lee, J., Broughton, R. M., et al. (2006). Synthesis and antimicrobial applications of 5,5′-ethylenebis 5-methyl-3-(3-triethoxysilylpropyl)hydantoin. *Biomaterials*, *27*, 4825–4830.

Buonocore, G. G., Del Nobile, M. A., Panizza, A., Bove, S., Battaglia, G., & Nicolais, L. (2003). Modeling the lysozyme release kinetics from antimicrobial films intended for food packaging applications. *Journal of Food Science*, *68*, 1365–1370.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection*, *67*, 833–848.

Cao, Z., & Sun, Y. (2008). N-halamine-based chitosan: Preparation, characterization, and antimicrobial function. *Journal of Biomedical Materials Research Part A*, *85*, 99–107.

Chen, M. C., Yeh, G. H. C., & Chiang, B. H. (1996). Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. *Journal of Food Processing and Preservation*, *20*, 279–290.

Chen, X.-G., & Park, H.-J. (2003). Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions. *Carbohydrate Polymers*, *53*, 355–359.

Chen, Z., & Sun, Y. (2006). N-halamine-based antimicrobial additives for polymers: Preparation, characterization, and antimicrobial activity. *Industrial and Engineering Chemistry Research*, *45*, 2634–2640.

de Moura, M. R., Mattoso, L. H. C., & Zucolotto, V. (2012). Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles and their use as active food packaging. *Journal of Food Engineering*, *109*, 520–524.

Du, W. X., Olsen, C. W., Avena-Bustillos, R. J., McHugh, T. H., Levin, C. E., & Friedman, M. (2008). Antibacterial activity against *E. coli* O157: H7, physical properties, and storage stability of novel carvacrol-containing edible tomato films. *Journal of Food Science*, *73*, M378–M383.

Fayaz, A. M., Balaji, K., Girilal, M., Kalaichelvan, P. T., & Venkatesan, R. (2009). Mycobased synthesis of silver nanoparticles and their incorporation into sodium alginate films for vegetable and fruit preservation. *Journal of Agricultural and Food Chemistry*, *57*, 6246–6252.

Haro, M., Giner, B., Lafuente, C., Lopez, M. C., Royo, F. M., & Cea, P. (2005). Proton sponge and fatty acid interactions at the air–water interface. Thermodynamic, spectroscopic, and microscopic study. *Langmuir*, *21*, 2796–2803.

Jiang, X., Cai, K., Zhang, J., Shen, Y., Wang, S., & Tian, X. (2011). Synthesis of a novel water-soluble chitosan derivative for flocculated decolorization. *Journal of Hazardous Materials*, *185*, 1482–1488.

Kocer, H. B., Cerkez, I., Worley, S. D., Broughton, R. M., & Huang, T. S. (2011). Polymeric antimicrobial N-halamine epoxides. *Acs Applied Materials & Interfaces*, *3*, 2845–2850.

Kumar, S., Koh, J., Kim, H., Gupta, M. K., & Dutta, P. K. (2012). A new chitosan–thymine conjugate: Synthesis, characterization and biological activity. *International Journal of Biological Macromolecules*, *50*, 493–502.

Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J., & Bigger, S. W. (2011). Essential oils and their principal constituents as antimicrobial agents for synthetic packaging films. *Journal of Food Science*, *76*, R164–R177.

Li, L.-H., Deng, J.-C., Deng, H.-R., Liu, Z.-L., & Li, X.-L. (2010). Preparation, characterization and antimicrobial activities of chitosan/Ag/ZnO blend films. *Chemical Engineering Journal*, *160*, 378–382.

Li, X., Xing, Y., Jiang, Y., Ding, Y., & Li, W. (2009). Antimicrobial activities of ZnO powder-coated PVC film to inactivate food pathogens. *International Journal of Food Science and Technology*, *44*, 2161–2168.

Liang, J., Chen, Y., Ren, X., Wu, R., Barnes, K., Worley, S. D., et al. (2007). Fabric treated with antimicrobial N-halamine epoxides. *Industrial & Engineering Chemistry Research*, *46*, 6425–6429.

Lin, J., Cammarata, V., & Worley, S. D. (2001). Infrared characterization of biocidal nylon. *Polymer*, *42*, 7903–7906.

Lu, G. Q., Wu, D. C., & Fu, R. W. (2007). Studies on the synthesis and antibacterial activities of polymeric quaternary ammonium salts from dimethylaminoethyl methacrylate. *Reactive & Functional Polymers*, *67*, 355–366.

Luo, J., & Sun, Y. (2008). Acyclic N-halamine coated Kevlar fabric materials: Preparation and biocidal functions. *Industrial & Engineering Chemistry Research*, *47*, 5291–5297.

Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*, 995–1012.

Muzzarelli, R. A. A., Tarsi, R., Filippini, O., Giovanetti, E., Biagini, G., & Varaldo, P. E. (1990). Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrobial Agents and Chemotherapy*, *34*, 2019–2023.

Muzzarelli, R. A., Ilari, P., & Petrarulo, M. (1994). Solubility and structure of N-carboxymethylchitosan. *International Journal of Biological Macromolecules*, *16*, 177–180.

Ren, X., Kocer, H. B., Kou, L., Worley, S. D., Broughton, R. M., Tzou, Y. M., et al. (2008). Antimicrobial polyester. *Journal of Applied Polymer Science*, *109*, 2756–2761.

Ren, X., Kocer, H. B., Worley, S. D., Broughton, R. M., & Huang, T. S. (2009). Rechargeable biocidal cellulose: Synthesis and application of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione. *Carbohydrate Polymers*, *75*, 683–687.

Sajomsang, W., Gonil, P., & Tantayanon, S. (2009). Antibacterial activity of quaternary ammonium chitosan containing mono or disaccharide moieties: Preparation and characterization. *International Journal of Biological Macromolecules*, *44*, 419–427.

Sajomsang, W., Ruktanonchai, U. R., Gonil, P., & Warin, C. (2010). Quaternization of N-(3-pyridylmethyl) chitosan derivatives: Effects of the degree of quaternization, molecular weight and ratio of N-methylpyridinium and N,N,N-trimethyl ammonium moieties on bactericidal activity. *Carbohydrate Polymers*, *82*, 1143–1152.

Seydim, A. C., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Research International*, *39*, 639–644.

Sikong, L., Kongreong, B., Kantachote, D., & Sutthisripok, W. (2010). Inactivation of salmonella typhi using $Fe^{(3+)}$ doped $TiO(2)/3SnO_{(2)}$ photocatalytic powders and films. *Journal of Nano Research*, *12*, 89–97.

Sun, Y. Y., & Sun, G. (2001). Novel regenerable N-halamine polymeric biocides. I. Synthesis, characterization, and antibacterial activity of hydantoin-containing polymers. *Journal of Applied Polymer Science*, *80*, 2460–2467.

Tankhiwale, R., & Bajpai, S. K. (2012). Preparation, characterization and antibacterial applications of ZnO-nanoparticles coated polyethylene films for food packaging. *Colloids and Surfaces B-Biointerfaces*, *90*, 16–20.

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2010). Preparation and physicochemical evaluation of chitosan/poly(vinyl alcohol)/pectin ternary film for food-packaging applications. *Carbohydrate Polymers*, *79*, 711–716.

Worley, S. D., Chen, Y., Wang, J. W., & Wu, R. (2005). Novel N-halamine siloxane monomers and polymers for preparing biocidal coatings. *Surface Coatings International Part B-Coatings Transactions*, *88*, 93–99.

Worley, S. D., Li, F., Wu, R., Kim, J., Wei, C. I., Williams, J. F., et al. (2003). A novel N-halamine monomer for preparing biocidal polyurethane coatings. *Surface Coatings International Part B-Coatings Transactions*, *86*, 273–277.

Xu, T., Xin, M., Li, M., Huang, H., & Zhou, S. (2010). Synthesis, characteristic and antibacterial activity of N,N,N-trimethyl chitosan and its carboxymethyl derivatives. *Carbohydrate Polymers*, *81*, 931–936.

Yang, T.-C., Chou, C.-C., & Li, C.-F. (2005). Antibacterial activity of N-alkylated disaccharide chitosan derivatives. *International Journal of Food Microbiology*, *97*, 237–245.